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Biochemical Properties of Inducible β -Lactamases Produced from *Xanthomonas maltophilia*

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Four different β -lactamases have been found in several strains of *Xanthomonas maltophilia* isolated from blood cultures during 1984 to 1991 at the Edinburgh Royal Infirmary. One was a metallo- β -lactamase with predominantly penicillinase activity and an isoelectric point of 6.8. Its molecular size as determined by gel filtration was 96 kDa but was only 26 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), suggesting a tetramer of four equal subunits. The enzyme hydrolyzed all classes of β -lactams except the monobactam aztreonam. This enzyme was not inhibited by potassium clavulanate or BRL 42715 but was inhibited by *p*-chloromercuribenzoate, mercuric chloride, and EDTA. The β -lactamase was unstable in 50 mM sodium phosphate buffer (pH 8.0) but stable in 50 mM Tris HCl (pH 8.0). The other β -lactamases focused as a series of different isoelectric points, ranging from pI 5.2 to 6.6. Together, these enzymes exhibited a broad spectrum of activity, hydrolyzing most classes of β -lactams but not imipenem or aztreonam. Their molecular size was 48 kDa by Sephadex gel filtration and 24 kDa by SDS-PAGE, indicating that they were enzymes consisting of two equal subunits. They were inhibited by *p*-chloromercuribenzoate, mercuric chloride, potassium clavulanate, and BRL 42715 but not EDTA. This study demonstrated that *X. maltophilia* produces more than just the L1 and L2 β -lactamases.

Xanthomonas maltophilia is the only member of the genus *Xanthomonas* that is pathogenic to humans. This species rests uneasily within this genus, however, and a recent report has proposed that it be placed in a new genus, which includes a single species, *Stenotrophomonas maltophilia* (24). They are glucose-nonfermenting, gram-negative bacilli and are increasingly recognized as an important opportunistic pathogen, often affecting patients with lowered defense mechanisms (12, 21, 22, 29). The species is commonly resistant to a wide range of β -lactams as well as other classes of antimicrobial agents (11, 15, 22). This resistance has been attributed to the interplay between outer membrane impermeability (20) and the production of two potent β -lactamases. L1, a metallo- β -lactamase (31), and L2, described as an unusual cephalosporinase (30), hydrolyze virtually the entire spectrum of β -lactams. It has been assumed that all strains produced the L1 and L2 enzymes. However, recent studies have shown an unexpected heterogeneity among β -lactamases produced by this species (9, 19). Both studies reported several β -lactamases differentiated by their isoelectric points. In the study by Cullmann and Dick (9), none of the strains examined produced more than one β -lactamase. In this study, we report the biochemical properties of β -lactamases produced by seven strains of *X. maltophilia* isolated from blood cultures at the Edinburgh Royal Infirmary during 1984 to 1991.

(Part of this work was presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy [25].)

MATERIALS AND METHODS

Bacterial strains. Seven strains of *X. maltophilia* were examined. All strains were isolated from blood cultures taken at the Edinburgh Royal Infirmary during 1984 to 1991. They were stored as lyophilized cultures until required. All strains were identified by the API 20NE identification strip.

Antimicrobial agents. The following antibiotics were tested and obtained from the following companies: penicillin and ampicillin from Sigma Chemical Co. (Dorset, England), BRL 42715 and potassium clavulanate from SmithKline Beecham (Welwyn Garden City, England), piperacillin from Lederle Laboratories (Gosport, England), imipenem from Merck Sharp & Dohme (Hoddesdon, England), ceftazidime and cefuroxime from Glaxo Laboratories Ltd. (Uxbridge, England), cefotaxime and gentamicin from Roussel Laboratories Ltd. (Uxbridge, England), azlocillin and ciprofloxacin from Bayer (Newbury, England), and aztreonam from Bristol-Myers Squibb (Hounslow, England).

Antimicrobial susceptibility tests. MICs were determined by the agar dilution method. All assays were performed on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, England). The inoculum used, approximately 10^4 CFU/ml, was applied with a Denley multipoint inoculator (Billinghurst, England). Plates were incubated overnight at 37°C. Each MIC was determined as the lowest concentration of antibiotic that inhibited visible growth.

IEF. Samples were applied to a polyacrylamide gel containing ampholines with a pH range of 3.5 to 10.6 or a 1:1 mixture of pH 3.5 to 10.6 and pH 4 to 6 as previously described (18). Isoelectric focusing (IEF) was carried out at 4°C at 1 W (constant), 500 V (limiting), and 20 mA (limiting) for 18 h. Gels were quantified with pI standard markers (BDH Ltd., Poole, England) and stained by overlaying the gels with filter paper soaked in nitrocefin (1 mM) either directly or after a 30-s overlay with various inhibitors (1 mM potassium clavulanate, 1 mM aztreonam, 1 mM cloxacillin, 1 mM EDTA, and 100 μ M BRL 42715) soaked in filter paper as previously described (2).

Assessment of inducibility of β -lactamase. Ten-milliliter volumes of Mueller-Hinton broth were grown overnight at 37°C with continuous shaking. One milliliter of overnight culture was added to 9 ml of prewarmed Mueller-Hinton broth and incubated for a further 90 min. Either cefotaxime or

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TABLE 1. Susceptibilities of *X. maltophilia* isolates to various antibiotics

Strain	Yr isolated	MIC ($\mu\text{g/ml}$) ^a									
		AMP	PIP	AZL	CXM	CTX	CAZ	IMP	AZM	CIP	GEN
5B105	1984	>256	>256	>256	>256	128	>256	256	>256	4	64
6B52	1985	>256	128	16	>256	128	256	128	>256	8	128
6B133	1985	>256	>256	>256	>256	128	>256	>256	>256	8	128
6B295	1985	>256	>256	>256	>256	128	>256	256	>256	4	64
7B78	1986	>256	64	16	>256	64	128	256	256	4	4
12B286	1991	>256	128	32	>256	128	128	64	>256	4	256
12B346	1991	>256	256	256	>256	256	>256	128	>256	2	32

^a Abbreviations: AMP, ampicillin; PIP, piperacillin; AZL, azlocillin; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; AZM, aztreonam; CIP, ciprofloxacin; GEN, gentamicin.

imipenem was then added at one-fourth the MIC. Control flasks with no added inducer were established. After incubation for a further 2 h, cells were harvested and washed and the β -lactamase was released by sonication; the procedure was repeated after 4 h. Enzyme activity was measured by UV spectrophotometry. Nitrocefin (100 μM) was used as the test substrate. Protein estimation was measured as previously described (34).

Large-scale preparation of β -lactamases. Mueller-Hinton broths (100 ml) were inoculated with the strain under study and grown overnight with shaking at 37°C. Cultures were then added to 900 ml of the same prewarmed broth, and incubation continued under the same conditions for 90 min. For induction, imipenem was then added at one-fourth the MIC. Incubation was continued for a further 3 h. After 3 h, bacterial cells were harvested by centrifugation at 4°C. The pellet was washed in 50 mM Tris HCl buffer (pH 8.0) and recentrifuged. The pellet was resuspended in 4 ml of the same buffer and disrupted by ultrasonication (8 μm for 1 min, three times) (MSE Soniprep 150; MSE Instruments, Crawley, United Kingdom) with constant cooling in an ice water bath. The preparation was partially purified by passing it through a Sephadex G-150 column (2 cm^2 by 90 cm) (Pharmacia, Uppsala, Sweden) previously equilibrated with 50 mM Tris HCl buffer (pH 8.0) containing 0.1 mM zinc sulfate. Samples were eluted with the same buffer with a flow rate of 12 ml/h. β -Lactamase activity was detected with the chromogenic cephalosporin nitrocefin. The molecular mass on this column was determined as previously described (1). The molecular weight standards were alcohol dehydrogenase (150,000), bovine serum albumin (66,000), and cytochrome *c* (12,384).

Purification of β -lactamase by electrodialysis. Five hundred microliters of the partially purified β -lactamase solution prepared from *X. maltophilia* 5B105 was applied to a preparative IEF gel containing pH 3.5 to 10.6 and pH 4 to 6 ampholines in a 1:1 ratio. After focusing as described above, a 1-cm-wide strip of filter paper soaked in nitrocefin solution was placed along either side of the gel from the anode to the cathode to determine the position of the focused β -lactamases. The β -lactamase bands were then excised from the gel and placed into a dialysis sack with a minimal amount of 50 mM sodium phosphate buffer (pH 7.0). The dialysis sack was placed in the cathode reservoir of a Mini Sub Cell (Bio-Rad Laboratories Ltd., Hemel Hempstead, England) previously filled with 50 mM sodium phosphate buffer (pH 7.0). A charge of 150 V was applied to the sack for 10 min. The dialysis sack was then removed, and the polyacrylamide gel discarded (28). The remaining purified β -lactamase preparation was reappplied to an IEF as described above.

SDS-free (native) PAGE. Sodium dodecyl sulfate (SDS)-free

(native) polyacrylamide gel electrophoresis (PAGE) was performed on the Phastsystem (Pharmacia). The buffer system used on the strips was 0.88 M L-alanine–0.25 M Tris (pH 8.8). Buffer strips were made of 2% agarose IEF. Fractions eluted from the G-150 column that contained all β -lactamase bands (as determined by IEF) were pooled and concentrated to 5 ml in a centrprep 10 concentrator (Amicon, Danvers, Mass.). Four microliters of this solution was applied to a Phastgel homogenous 12.5 minigel. The separations were run as recommended by the manufacturer. β -Lactamase activity was visualized with nitrocefin as described above.

Determination of subunit size. The mass of the β -lactamase subunit was also estimated by SDS-PAGE (14). β -Lactamase extracts were treated with 5.0% β -mercaptoethanol and 2.5% SDS at 100°C for 5 min before electrophoresis on a Phastgel gradient 10 to 15 minigel according to the manufacturer's instructions. After electrophoresis, gels were incubated for 4 h in 50 mM Tris HCl buffer (pH 8.0) containing 1% Triton X-100 and 0.1 mM zinc sulfate to obtain a renaturation of enzymatic activity as described previously (17). After renaturation was complete, visualization of β -lactamase activity was obtained by staining with nitrocefin as described above. Low-molecular-weight protein standards (Bio-Rad Laboratories) were run simultaneously under the same conditions and stained with

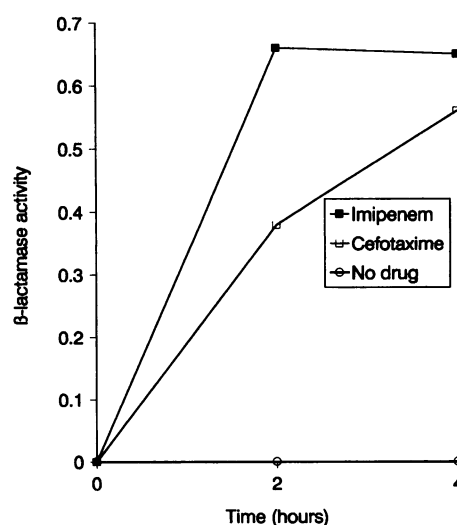


FIG. 1. Kinetics of β -lactamase induction for *X. maltophilia* 5B105. β -Lactamase activity is expressed as \log_{10} nanomoles of nitrocefin hydrolyzed per minute per milligram of protein.

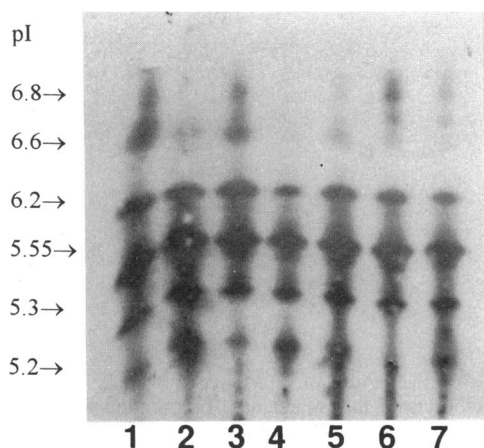


FIG. 2. IEF patterns of β -lactamases from seven strains of *X. maltophilia* isolated from blood cultures during 1984 to 1991. Lane 1, 5B105; lane 2, 6B52; lane 3, 6B133; lane 4, 6B295; lane 5, 7B78; lane 6, 12B286; lane 7, 12B346.

Coomassie brilliant blue R250. The molecular weight standards were transposed over the gel with the renatured β -lactamases for the molecular masses to be ascertained.

β -Lactamase assays. Assays of β -lactamase activity were performed at 37°C on a Perkin-Elmer λ 2 spectrophotometer with freshly prepared antibiotic solutions in either 50 mM Tris HCl buffer (pH 8.0) or 50 mM sodium phosphate buffer (pH 7.0) at the wavelength of maximal absorbance for the β -lactam ring of each drug over a 5-min period. The following wavelengths were used: 238 nm for penicillin and ampicillin, 236 nm for carbenicillin, 260 nm for cefuroxime and ceftazidime, 265 nm for cefotaxime, 240 nm for azlocillin, 255 nm for cephaloridine, and 299 nm for imipenem. Maximum rate of hydrolysis (V_{max}) and K_m values were derived by linear regression analysis of Lineweaver-Burk plots of initial velocity data at different substrate concentrations.

Inhibition studies. A solution of enzyme was preincubated with inhibitor for 5 min at 37°C in either 50 mM Tris HCl buffer (pH 8.0) or 50 mM sodium phosphate buffer (pH 7.0). The remaining enzymatic activity was assayed spectrophotometrically with cephaloridine (100 μ M), imipenem (100 μ M), or nitrocefin (100 μ M) as the test substrate.

RESULTS

Antimicrobial susceptibilities. Table 1 shows the results of antimicrobial susceptibilities. All seven strains examined were resistant to almost all of the β -lactams tested, including imipenem. Only two strains (6B52 and 7B78) were susceptible to azlocillin (MIC, 16.0 μ g/ml). All isolates were also resistant to gentamicin (breakpoint, 1.0 μ g/ml [4]). Ciprofloxacin was the most effective agent of all compounds tested, with MICs for five of the seven strains tested either at or below the recommended breakpoint level (4 μ g/ml) (4).

Inducibility of β -lactamase production. The kinetics of β -lactamase induction for strain 5B105 are shown in Fig. 1. The test substrate was nitrocefin. When imipenem or cefotaxime was added as an inducer, β -lactamase activity increased sharply after less than 1 h. Imipenem was a more potent inducer of β -lactamase than cefotaxime was. No discernible β -lactamase activity was detected in disrupted cells without the prior addition of an inducer. This result demonstrated that all β -lactamase activity was inducible. No enzyme activity was

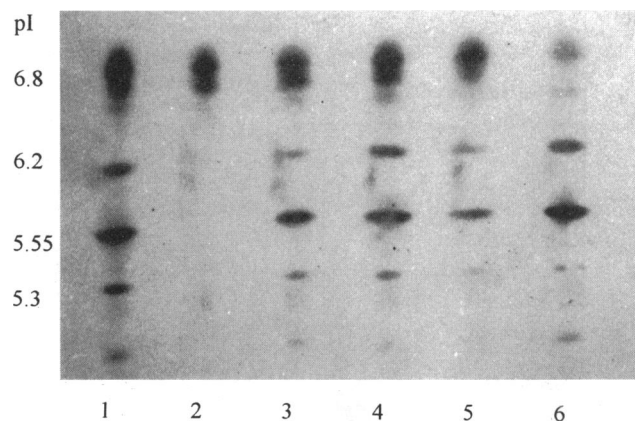


FIG. 3. IEF patterns of *X. maltophilia* β -lactamases overlaid with various inhibitors. Lane 1, control; lanes 2 to 6, overlaid with 100 μ M BRL 42715, 1 mM aztreonam, 1 mM cloxacillin, 1 mM clavulanate, and 1 mM EDTA, respectively.

detected in the culture filtrate, indicating that the β -lactamase was intracellular.

IEF. β -Lactamase preparations from all seven strains exhibited identical IEF patterns and revealed the presence of at least four main bands of β -lactamase activity (pI 6.8, 6.2, 5.55, and 5.3) and two minor bands (pI 5.2 and 6.6) (Fig. 2). *X. maltophilia* 5B105 was selected as representative and examined in more detail.

Polyacrylamide gels were overlaid with various inhibitors to further elucidate the nature of these β -lactamases (Fig. 3). The β -lactamase band of pI 6.8 was inhibited by overlaying the gel with 1.0 mM EDTA prior to staining, whereas all other bands were unaffected. Conversely, the other β -lactamase bands were completely eliminated by the overlay of BRL 42715 and partially inhibited by potassium clavulanate. Aztreonam and cloxacillin overlays appeared to have no significant effect on any of the β -lactamase bands.

Gel filtration of crude β -lactamase. Spectrophotometric analysis of the fractions eluted from the Sephadex G-150 column and subsequent IEF (Fig. 4) revealed the presence of at least two distinct enzymes. The β -lactamase with a pI of 6.8 eluted first from the column and hydrolyzed imipenem as well as nitrocefin; the other β -lactamase bands eluted from the column at the same rate and hydrolyzed nitrocefin but not imipenem. The molecular masses of the enzymes were calcu-

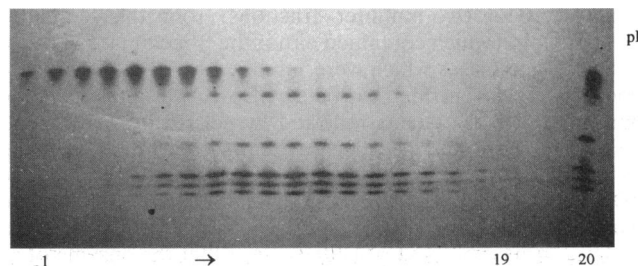


FIG. 4. IEF patterns of β -lactamases from *X. maltophilia* 5B105 eluted from a Sephadex G-150 column. Lanes 1 to 19, 15 μ l of every second fraction eluted from a Sephadex G-150 column (fractions 52 to 88) applied to the gel; lane 20, concentrated β -lactamase preparation of *X. maltophilia* 5B105.

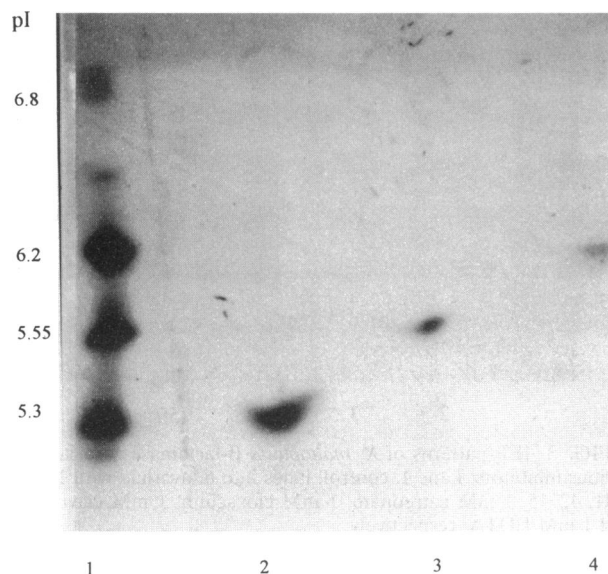


FIG. 5. IEF of β -lactamases from *X. maltophilia* 5B105 purified by electro dialysis. Lane 1, original β -lactamase preparation from 5B105; lanes 2 to 4, β -lactamase bands (pI 5.3, 5.55, and 6.2, respectively) purified by electro dialysis.

lated as 96 and 48 kDa, respectively, by comparing peak activity with the standard proteins.

In preliminary experiments, the β -lactamase of pI 6.8 was found to be unstable in 50 mM sodium phosphate buffer (pH 7.0) at 37°C and 80% of the activity was lost after 5 min of incubation in the buffer. The enzyme was stable in 50 mM Tris HCl with an optimum pH of 8.0. Therefore, all assays on the β -lactamase of pI 6.8 were performed with this buffer. The other β -lactamases were stable in phosphate buffer, and assays of these enzymes were performed with 50 mM sodium phosphate buffer (pH 7.0).

Purification of β -lactamase by electro dialysis. Purification of the three main β -lactamase bands (pI 5.3, 5.55, and 6.2) that were eliminated by BRL 42715 was performed by electro dialysis. Each band was excised from the gel, repurified, applied to an IEF gel, and re-separated (Fig. 5). Each preparation focused as a single band at exactly the same pI to which it had originally migrated, indicating that each β -lactamase was a discrete β -lactamase rather than a satellite band.

Native PAGE. Native PAGE of the concentrated β -lactamase preparation from the Sephadex G-150 gel column revealed the presence of four distinct bands of activity, compatible with the concept of four β -lactamases (Fig. 6).

SDS-PAGE. Two-milliliter fractions from the Sephadex G-150 column which contained either the β -lactamase of pI 6.8 or the β -lactamases which were inhibited by BRL 42715 were pooled and concentrated to 200 μ l. SDS-PAGE analysis of the partially purified extracts followed by gel renaturation treatment (Fig. 7) showed in each case the presence of a single band of β -lactamase activity, with an apparent molecular mass of 26 kDa for the enzyme of pI 6.8, while the other β -lactamase bands showed the presence of a single protein with an apparent molecular mass of 24 kDa. Data suggest that the enzyme of pI 6.8 is a tetramer of four subunits which are very similar in molecular mass. Between them, the other β -lactamases exhibited a single band of β -lactamase activity by SDS-PAGE, indicating dimetric enzymes consisting of two subunits of similar size.

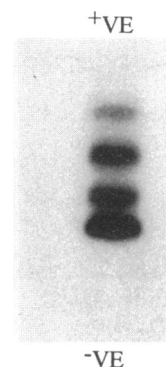


FIG. 6. Native PAGE of β -lactamases from *X. maltophilia* 5B105. Four microliters of the β -lactamase preparation was applied at the cathode. +VE, anode; -VE, cathode.

Hydrolysis of β -lactam antibiotics. Assays of β -lactamase hydrolysis of various β -lactams were performed with partially purified extracts from the G-150 column on the β -lactamase of pI 6.8. It was clearly evident at this stage that the β -lactamases of lower pI (<6.8) could not be separated, and they were subsequently pooled. For convenience, these β -lactamases are hereafter designated XM-A and XM-B, respectively (Table 2).

XM-A was primarily a penicillinase and also readily hydrolyzed imipenem. It was much less active against all classes of cephalosporins, although its affinities (low K_m values) for cephalosporins were higher than those for penicillins and imipenem. Aztreonam was not hydrolyzed. The XM-B enzymes exhibited a broad substrate profile, hydrolyzing an extensive range of β -lactams but not imipenem, ceftazidime, or aztreonam. Their affinities were higher for cephalosporins than for penicillins.

Inhibitors. Table 3 shows the effect of inhibitors on the activities of enzymes. The treatment of XM-A with EDTA resulted in the complete inhibition of enzymatic activity. This inhibition was completely reversed after dialysis against 50 mM Tris HCl (pH 8.0) for 30 min and the addition of 0.1 mM zinc sulfate to the reaction mixture. Enzymatic activity was partially inhibited by mercuric chloride and *p*-chloromercuribenzoate (PCMB) but not by either potassium clavulanate or BRL 42715 (50% inhibitory doses of >50 and >10 μ M, respective-

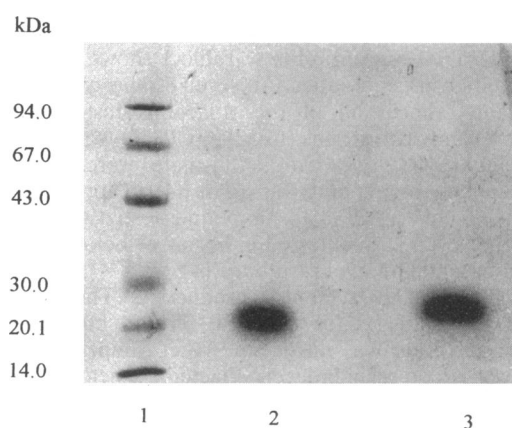


FIG. 7. SDS-PAGE of the β -lactamases from *X. maltophilia* 5B105. Lane 1, molecular mass standards; lane 2, β -lactamase bands of XM-B; lane 3, β -lactamase band of XM-A.

TABLE 2. Hydrolysis of β -lactam antibiotics by XM-A and XM-B β -lactamases from *X. maltophilia* 5B105

Substrate	XM-A			XM-B		
	V_{\max}^a	Relative V_{\max}^b	K_m (mM)	V_{\max}^a	Relative V_{\max}^b	K_m (mM)
Penicillin	14.3	100	0.6	1.2	100	0.108
Ampicillin	5.71	39.9	0.34	2.3	191.6	0.22
Carbenicillin	2.0	13.9	0.67	0.45	37.5	0.55
Azlocillin	5.5	38.4	0.24	1.25	104	0.22
Cephaloridine	0.09	0.6	0.22	1.8	150	0.05
Cefuroxime	0.66	4.6	0.133	0.28	23.3	0.076
Cefotaxime	0.303	2.1	0.05	0.19	15.8	0.08
Ceftazidime	0.03	0.2	0.09	NMH ^c		
Imipenem	3.33	23.3	0.25	NMH		
Aztreonam	NMH			NMH		

^a Expressed as micromoles of substrate hydrolyzed per minute per milliliter of enzyme solution.

^b Relative to the V_{\max} of penicillin set at 100%.

^c NMH, no measurable hydrolysis.

ly). The XM-B enzymes were not affected by the addition of 0.1 mM EDTA but were inhibited by both 1 mM potassium clavulanate and 100 μ M BRL 42715 (50% inhibitory doses of 0.16 and <0.001, respectively). The enzymatic activity of XM-B was completely inhibited by the addition of 0.5 mM PCMB and 0.5 mM mercuric chloride (Table 3).

DISCUSSION

There have been few reports that have characterized the β -lactamases encoded by *X. maltophilia* in detail; it has been generally assumed that all isolates produce two β -lactamases, L1 and L2. Recent work, however, has revealed the heterogeneity of β -lactamase production by strains of *X. maltophilia*. These enzymes have not been characterized in full but have been differentiated only by virtue of their isoelectric points (9, 19).

All enzymatic activity observed was inducible. The production of two inducible β -lactamases is unusual, although it has previously been reported for strains of *Aeromonas* spp. (13), *Bacillus cereus* (10), and *Yersinia enterocolitica* (8). Some *Aeromonas* spp. have been reported to produce up to three different β -lactamases (32).

The multiple bands of β -lactamase activity observed with the strains examined in this report were difficult to purify to homogeneity. Repeated attempts to purify each band of β -lactamase activity were unsuccessful despite the utilization of ion-exchange chromatography with different pH buffers (i.e., above and below the pI of the β -lactamase bands). Not only did the distinct bands of β -lactamase fail to separate but the resulting enzymatic activity of the eluate was very poor. This technique was especially detrimental to the activity of the

XM-A enzyme. However, the β -lactamase activity could be separated by gel filtration into two fractions, XM-A and XM-B.

Polyacrylamide gel overlays with various inhibitors demonstrated that XM-A was inhibited by EDTA but not by BRL 42715 (a powerful inhibitor of serine-active-site β -lactamases [7]), indicating that this enzyme was distinct from the other bands of activity. Inhibitor overlays also demonstrated that the XM-B enzymes were inhibited by both BRL 42715 and potassium clavulanate.

The differences in the pIs of the XM-B bands suggest that they may have undergone amino acid substitution or addition away from the active site of the enzyme and may physically differ in their primary structure. Purification by electrodialysis and subsequent IEF demonstrated that each preparation focused as a single band of activity at exactly the same pI to which it had originally migrated. Its small yield of enzyme made this technique unsuitable for the purification of enzyme for further studies. Native PAGE of the concentrated partially purified β -lactamase preparation revealed the presence of four distinct bands of activity. Separated by gel filtration, all the bands that were inhibited by BRL 42715 were eluted at the same rate, with an apparent molecular mass of 48 kDa. When the molecular mass was estimated by SDS-PAGE and subsequent renaturation of β -lactamase activity, only one band with a molecular mass of 24 kDa was observed, suggesting a dimeric enzyme. It is improbable that an organism would encode three or more β -lactamases with identical molecular masses and subunit conformation unless they were closely related, like the TEM enzymes, and in parallel with the TEM β -lactamases, the differences in pIs arise from a few amino acid substitutions. These results indicated that the β -lactamase activity of XM-B was the result of at least three distinct enzymes and not of satellite bands. Unfortunately, the identical molecular masses of these enzymes and the similarities of their pIs made separation of these enzymes impossible, despite employing cation- and anion-exchange chromatography.

The XM-A enzyme was very similar in physical and biochemical profile to the L1 enzyme. It was unstable in sodium phosphate buffer. The plasmid-mediated metallo- β -lactamase from *Pseudomonas aeruginosa* is also unstable in phosphate buffer (35). The catalytic activity of the XM-A enzyme was dependent on Zn^{2+} for activity. Although the restoration of activity with other metal ions was not attempted in this study, results suggest that the enzyme is a metallo- β -lactamase. Both enzymes share similar pIs (L1, pI 6.9). In parallel with the L1 enzyme, the XM-A enzyme also exists as a tetramer in the active state (molecular mass, 96 kDa), with a subunit molecular mass of 26 kDa. The inhibitory profiles of both enzymes are very similar. Both are insensitive to potassium clavulanate but susceptible to mercuric chloride. Iaconis and Sanders (13) reported that the L1-producing strain they examined was inhibited by PCMB (20%), whereas in 1982, Saino et al. (31) reported no inhibition by this compound. Under our test conditions, PCMB inhibited the enzymatic activity of the XM-A enzyme by 69%. The zinc-active sites of metallo- β -lactamases are thought to include a conserved cysteine residue (3); therefore, inhibition by PCMB would be expected. As with other metallo- β -lactamases (16), XM-A, although exhibiting predominantly penicillinase activity, also hydrolyzed a broad range of β -lactams including imipenem but not the monobactam aztreonam.

The other enzymes described in this report, XM-B, although differing particularly in their isoelectric points from that of the L2 enzyme described by Saino et al. (30) (pI 8.4), shared some similarities with this enzyme. However, it should be noted that

TABLE 3. Effect of various inhibitors and ions on the activity of the XM-A and XM-B β -lactamases

Inhibitor	Concn (mM)	% Inhibition of:	
		XM-A ^a	XM-B ^b
PCMB	0.5	69	100
Mercuric chloride	0.5	70	100
EDTA	0.1	100	0

^a Nitrocefin (100 μ M) was used as the test substrate.

^b Cephaloridine (100 μ M) was used as the test substrate.

the results obtained with these combined enzymes should be treated minimally. We have shown that these enzymes have distinct physical properties (pIs); nevertheless, it has not been unequivocally shown that all these enzymes react in the same manner. The inhibitor profile of XM-B was similar to that reported for the L2 enzyme. All of these enzymes were inhibited by clavulanate, PCMB, and mercuric chloride. EDTA had no effect on the enzymatic activity of XM-B, indicating that XM-B does not require a divalent metal ion for catalytic activity. The inhibition by BRL 42715 suggests these enzymes are serine-active-site β -lactamases. The molecular mass of these enzymes was 48 kDa by gel filtration, whereas that of their subunit form was estimated to be 24 kDa by SDS-PAGE. The results show they are dimers in the native state, similar to the L2 enzyme (active-form molecular mass, 56 kDa; subunit molecular mass, 27 kDa). Together, the XM-B group of enzymes hydrolyzed a broad spectrum of antibiotics, including penicillins and cephalosporins, but not imipenem or aztreonam, whereas the L2 enzyme was primarily a cephalosporinase, with poor hydrolytic activity against penicillins.

Neither XM-A nor the XM-B group of enzymes was shown to hydrolyze aztreonam, although all the strains examined exhibited high levels of resistance to this drug. Another explanation of the high level of resistance to aztreonam may be the low permeability of the outer membrane of *X. maltophilia* to the influx of antibiotics (20).

Although the exact genetic locations of the genes which encode these β -lactamases have not been precisely determined, the inducibility of the enzymes would suggest that they are chromosomally mediated. Indeed, from this study and others (9, 27), there appears to be a paucity of plasmid-mediated β -lactamases in strains of *X. maltophilia*.

All β -lactamases are produced with a single inducer; this might imply that the enzymes share a common regulatory gene, although it is known that imipenem is an effective inducer for more than one β -lactamase (33).

We have shown that the XM-A enzyme shares biological and physiological properties with the L1 enzyme and clearly belongs in group 3 (metallo- β -lactamases [MET-N]) in the classification scheme of Bush (6); however, full sequencing is necessary for an absolute determination. The XM-B group of enzymes appears to be well placed in the Bush class 2b' (extended-broad-spectrum β -lactamases inhibited by clavulanic acid [EBS-Y]) (5); however, without cloning the individual genes into a suitable host and subsequently sequencing the enzymes, it is not possible to determine whether these enzymes have diverse biochemical profiles. The XM-A and XM-B enzymes may be common among strains of *X. maltophilia*. Cullmann and Dick (9) reported the presence of six distinct β -lactamases in 19 strains of *X. maltophilia*. On close scrutiny of the IEF polyacrylamide gel, there appear to be β -lactamases from strain 858 that are similar to those described in this report, although Cullmann and Dick do not describe these enzymes in their text. Recently, Payne et al. (27) reported the presence of enzymes with similar pIs in two strains of *X. maltophilia*. It appears that within the species *X. maltophilia*, a number of different β -lactamases which differ from the β -lactamases of most other genera are produced.

Livermore (16) has classified carbapenemases as efficient or inefficient, depending on whether they confer high or low levels of carbapenem resistance on the host organism. High levels of carbapenem resistance have been found only in those organisms that are known to commonly produce a chromosomal metallo- β -lactamase. The metallo- β -lactamase of *X. maltophilia* is an example. There is also a frightening possibility that the chromosomal genes that encode the β -lactamases of this

species may be transposed onto conjugative plasmids. There have been recent reports of inefficient carbapenemases, although not transferable, from *Acinetobacter baumannii* (26), *Enterobacter cloacae* (23), and *Serratia marcescens* (36), which are not of the normal β -lactamase complement for the host species. The increased use of β -lactam drugs, particularly the newer carbapenem compounds, may facilitate the emergence of this organism as an important pathogen. If the genes encoding these enzymes acquire the capability to disseminate among the more common pathogens, it will further diminish the range of antibiotics available for use in cases of serious sepsis.

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